



Immunomodulatory activity of *Mangifera indica* L. fruits (cv Neelam)

Tanveer Naved, Javed Inam Siddiqui, S. H. Ansari*, Anis A. Ansari¹, Hayat M. Mukhtar

1. Department of AYUSH, Ministry of Health & Family Welfare, Govt. of India, Red Cross Building, New Delhi-110001.
Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110 062.

Abstract

Objective: To evaluate the immunomodulatory activities of ethanolic extract of *Mangifera indica* L. cultivar Neelam fruit pulp. **Materials and methods:** In the present study, the alcoholic extract of fruit pulp of *M. indica* has been investigated for its effect on cell mediated and humoral components of the immune system in mice. **Results:** Administration of test extract produced increase in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) in mice. **Conclusion:** *M. indica* fruit pulp showed statistically significant immunostimulant potential in mice.

1. Introduction

Immunomodulation is a procedure that can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as immunostimulatory drug which primarily implies stimulation of non-specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors.

Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence

both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world [1].

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the effect throughout the immune system [2].

Traditional Indian system of medicines like Siddha, Ayurveda and Unani have suggested means to increase the body's natural resistance

* Corresponding author
shansari189@rediffmail.com

to disease. A number of Indian medicinal plants and various 'rasayanas' have been claimed to possess immunomodulatory activity [3-6].

Mangifera indica L. (Anacardiaceae) is a medicinal plant claimed to possess number of therapeutic uses. Mangiferin (1,3,6,7 tetrahydroxy xanthone 2-glucopyranoside) has been reported to be present in various parts viz. leaves [7], fruits [8], stem bark [9,10], heartwood [11] and roots [12].

It has been reported that *M. indica* possess immunomodulatory activity *in vitro* [13]. However, there is paucity of scientific data on the *in vivo* immunomodulatory activity of fruit pulp. The objective of present investigation was to study the immunomodulatory activity of the alcoholic extracts of the fruit pulp.

2. Materials and methods

2.1 Plant material

Fruits of *M. indica* cultivar Neelam was purchased from local market-Okhla mandi and authenticated at the Taxonomy Division, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi-110 062. A voucher specimen was deposited in the laboratory of Pharmacognosy & Phytochemistry, Jamia Hamdard, New Delhi.

2.2 Preparation of the extract

Fresh fruit pulp was dried under shade and then subjected to Soxhlet extraction with 95% v/v ethanol for 8 h. The total alcoholic extract was concentrated and air dried to obtain yellowish brown viscous mass (yield 4.52% w/w).

2.3 Reagents

Medium RPMI 1640 was obtained from Sigma, and Alsever's solution, Normal solution were obtained from Novartis. Microtitration plates (96 U wells) were obtained from Laxbro, Pune, India.

2.4 Animals

Female Swiss Albino mice (25-30g) were used. They were procured from Central Animal House, Jamia Hamdard (173/CPCSEA), after approval under project number 131. They were maintained under standard environmental conditions and had free access to feed (Hindustan Lever, India) and tap water *ad libitum*.

2.5 Antigen

Fresh sheep blood was obtained from Avon sheep blood suppliers, Majeedia Hospital, Jamia Hamdard, New Delhi. Sheep red blood cells (SRBCs) were washed three times in normal saline and adjusted to a concentration of 0.1ml containing 1×10^8 cells for immunization and challenge.

2.6 Statistical analysis

Data were expressed as the mean $\pm$ the correspondent standard deviation (S.D.) and statistical analysis was carried out by employing Student's *t* - test.

3. Humoral antibody (HA) and delayed type hypersensitivity (DTH) response

3.1 Delayed type hypersensitivity [14]

Delayed sensitivity was induced in mice using sheep red blood cells (SRBC) as antigen in Alsever's solution [15]. Animals were primed with 1×10^8 SRBC (day 0) and challenged on day 5 with 0.1ml of 1×10^8 SRBC subcutaneously in the hind foot paw. The right hind paw received saline alone. During this period, the test extract was fed from day 1 to day 5. Test drug was administered at a dose of 100 mg/kg, 200mg/kg and 300mg/kg p.o. Paw thickness measurements were made with Baker's pocket thickness gauge at 0, 24, 48, 72 and 96 h after the challenge.

The difference in paw thickness was taken as a measure of delayed hypersensitivity. After measurement of paw thickness, blood from

Table 1
Effect of *M. indica* pulp alcoholic extracts on SRBC induced Delayed Type Hypersensitivity (DTH) in mice

Group	Treatment	Dose (mg/kg)	Mean foot pad thickness (mm) (Mean $\pm$ S.D.)
I	Control	—	0.211 $\pm$ 0.011
II	Test extract	100	0.235 $\pm$ 0.010**
III	Test extract	200	0.248 $\pm$ 0.011***
IV	Test extract	300	0.275 $\pm$ 0.010***

n=6 per group; results are expressed as mean $\pm$ S.D. Significant differences compared from control by Student's *t* - test are indicated below.

NS, non-significant; $p < 0.01^{**}$ (Comparison of I with II); $p < 0.001^{***}$ (Comparison of I with III); $p < 0.001^{***}$ (Comparison of I with IV); $p > 0.05$ NS (comparison of II of III); $p < 0.001^{***}$ (Comparison of II with IV); $p < 0.01^{**}$ (Comparison of III with IV)

each animal was collected in microcentrifuge tubes (Laxbro) by sacrificing the animal, and serum was separated and subjected to hemagglutinating antibody (HA).

3.2 Humoral antibody (HA) response

Mice were intraperitoneally immunised with 0.1ml of 1×10^8 SRBC on day 0. Blood samples were collected from individual animals on day 5. Antibody levels were determined by the hemagglutination technique [16].

Two-fold diluted sera in saline (25 μ l) were mixed with 25 μ l of 0.1% v/v SRBC suspension in microplates. The plates were incubated 37°C for 1 h and then inspected for hemagglutination. The highest dilution giving rise to macroscopic hemagglutination was taken as antibody titre. Antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1, and the mean ranks of different groups were compared for statistical significance.

3.3 Drug treatment

The 95%v/v alcoholic extract of *M. indica* (100-300 mg/kg p.o.) was administered as follows 9 days around sensitisation (days -3, -2, -1, 0, +1, +2, +3, +4, +5)

4. Results and discussion

The main objective of this study was to focus on the immunomodulatory activity in animal models. Control of disease by immunological means has two aspects, namely the development and improvement of the protective immune status of organism and the avoidance of undesired immunological side reactions. Modulation of the immune system by plant origin agents is emerging as a major area in scientific studies, especially in cases where undesired immunosuppression is the result of therapy.

In the present study, SRBC-induced delayed type hypersensitivity was used to assess the effect of *M. indica* on cell-mediated immunity. In the control animals, the +48 h and +72 h response was either equal or slightly more than the 0 h response, therefore, the peak oedema at +24 h was taken as a parameter for evaluating the reaction. The alcoholic extract (100-300 mg/kg, p.o.) produced a significant dose-related increased DTH in mice. (Table 1)

To evaluate the effect of *M. indica* on humoral response, its influence was observed on sheep erythrocyte-specific hemagglutination antibody titre in mice. The alcoholic extract

Table 2

Effect of *M. indica* pulp alcoholic extract on SRBC induced Humoral antibody titres (HA) in mice

Group	Treatment	Dose (mg/kg)	Hemagglutination antibody titres (Mean $\pm$ S.D.)
I	Control	—	5.76 $\pm$ 0.51
II	Test extract	100	6.53 $\pm$ 0.23**
III	Test extract	200	6.68 $\pm$ 0.16**
IV	Test extract	300	8.26 $\pm$ 0.37***

n=6 per group; results are expressed as mean $\pm$ S.D. Significant differences compared from control by Student's *t* - test are indicated below.

NS, non-significant ; $p < 0.01^{**}$ (Comparison of I with II) ; $p < 0.01^{**}$ (Comparison of I with III) ; $p < 0.001^{***}$ (Comparison of I with IV) ; $p > 0.05$ NS (comparison of II of III) ; $p < 0.001^{***}$ (Comparison of II with IV) ; $p < 0.001^{***}$ (Comparison of III with IV).

(100-300 mg/kg, p.o.) showed significant increased the production of circulating antibodies (Table 2). Therefore, the present study establishes the cellular and humoral immunomodulatory property of the alcoholic extract of *M. indica* as immunostimulatory agent *in vivo*.

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